Pharmacokinetics and bioavailability of orbifloxacin oral suspension in New Zealand White rabbits (Oryctolagus cuniculus)

Megan K. Watson DVM, MS
Luke A. Wittenburg DVM, PhD
Christine T. Bui MBA, DVM
Kira A. Jarosz BS
Daniel L. Gustafson PhD
Matthew S. Johnston VMD

OBJECTIVE
To evaluate the pharmacokinetics and bioavailability of 2 doses of orbifloxacin in rabbits.

ANIMALS
6 healthy purpose-bred adult female New Zealand White rabbits (Oryctolagus cuniculus).

PROCEDURES
Each of 3 rabbits received orbifloxacin at either 10 or 20 mg/kg, PO. Then, after a 1-week washout period, they received the same dose IV. Blood samples were collected from each rabbit at 0, 0.25, 0.5, 1, 2, 4, 6, 12, and 24 hours after drug administration. Plasma orbifloxacin concentration was measured with liquid chromatography–tandem mass spectrometry. Pharmacokinetic parameters were determined by noncompartmental analysis for data obtained following PO administration and noncompartmental and compartmental analyses for data obtained following IV administration.

RESULTS
Following oral administration, the mean ± SD peak plasma orbifloxacin concentration was 1.66 ± 0.51 µg/mL for rabbits administered the 10 mg/kg dose and 3.00 ± 0.97 µg/mL for rabbits administered the 20 mg/kg dose and was attained at 2 hours after drug administration. The mean ± SD half-life of orbifloxacin in plasma was 7.3 ± 1.1 hours for rabbits administered the 10 mg/kg dose and 8.6 ± 0.55 hours for rabbits administered the 20 mg/kg dose. Mean bioavailability was 52.5% for rabbits administered the 10 mg/kg dose and 46.5% for rabbits administered the 20 mg/kg dose.

CONCLUSIONS AND CLINICAL RELEVANCE
Results provided pharmacokinetic properties for 2 doses (10 mg/kg and 20 mg/kg) of orbifloxacin oral suspension in rabbits. Further studies are necessary to determine the protein-binding activity of orbifloxacin in rabbits before dosages for the treatment of common pathogens in this species are recommended. (Am J Vet Res 2015;76:946–951)
MIC antimicrobials typically only need to be administered once daily. Fluo-roquinolones are bactericidal with good activity against gram-negative bacteria and slightly less activity against aerobic gram-positive bacteria and certain Mycoplasma spp.1,5,10–12 The excretion properties of fluoroquinolones vary by species and, to our knowledge, have not yet been described for rabbits.

Enrofloxacin is the most commonly used fluoroquinolone in veterinary medicine, and the pharmacokinetics of this drug has been established for rabbits.5,13,14 Marbofloxacin has also been studied in rabbits.3 Unfortunately, for enrofloxacin or marbofloxacin to be orally administered to rabbits, they must be compounded into palatable liquid formulations, which results in unavoidable variations in the potency, stability, palatability, and concentration of individually compounded suspensions. Because of recent restrictions on and the complicated laws associated with compounding drugs from bulk active pharmaceutical ingredients, alternatives to compounded drugs are desirable for veterinarians who treat companion exotic and zoological species, and commercially available preparations are preferable in certain situations.15,16

Orbifloxacin is approved to treat skin, soft tissue, and urinary tract infections caused by aerobic gram-negative and gram-positive bacteria in dogs and cats and is commercially available as a 30 mg/mL suspension that, on the basis of anecdotal reports, is palatable to rabbits. It is administered once daily to dogs and cats. Although the pharmacokinetics of orbifloxacin have been determined for horses,17 which like rabbits are hindgut fermenters, information regarding the administration of orbifloxacin to exotic species is lacking. Results of 1 study18 indicate that therapeutic plasma concentrations of orbifloxacin were not achieved in rabbits when a single dose (5 mg/kg) of the drug was administered by an IM, IV, or SC route. The objective of the study reported here was to determine the pharmacokinetics and bioavailability of 2 doses (10 mg/kg and 20 mg/kg) of orbifloxacin following oral administration to domestic rabbits.

Materials and Methods

Animals
Six purpose-bred sexually intact female New Zealand White rabbits (Oryctolagus cuniculus) with body weights ranging from 3.54 to 4.07 kg were used in the study. All rabbits were determined to be healthy on the basis of physical examination results. Rabbits were housed at Colorado State University in a facility that was accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, where they were maintained in 12 hours of light and 12 hours of darkness daily. They were fed commercial rabbit pellets and grass hay and provided water ad libitum. All study procedures were approved by the Colorado State University Animal Care and Use Committee.

Study design
The study had a crossover design. Rabbits were randomly allocated to 2 treatment groups (3 rabbits/group) by use of a random number generator and administered the assigned dose (10 or 20 mg/kg) of orbifloxacin orally, then after a 1-week washout period, received the same dose IV. Each rabbit was individually weighed on a standard scale immediately prior to each administration of orbifloxacin to ensure accurate calculation of the volume of drug administered.

For oral administration, the assigned dose of a commercially available orbifloxacin oral suspension was administered by a syringe technique that consisted of wrapping each rabbit in a towel leaving only its head exposed and placing the tip of a 3-mL syringe in the diastema. The calculated volume (range, 1 to 3 mL) of orbifloxacin suspension was injected into the mouth in small aliquots, and the rabbits were allowed to swallow each amount before the next aliquot was administered. Following administration, each rabbit, the towel it was wrapped in, and the examination table were examined to ensure that 100% of the calculated volume of orbifloxacin was ingested.

Because a commercially available orbifloxacin solution was not available for IV administration, the orbifloxacin solution administered IV was prepared from microionized orbifloxacin powder provided by the manufacturer of the oral suspension. A certificate of authenticity provided with the powder stated that its purity was 99.3%. The orbifloxacin powder (1 g) was initially dissolved in 100 mL of a lactic acid solution then filtered through a 0.22-µm filter to prevent bacterial contamination (the manufacturer assured us that filtration through that size of filter would not affect the chemical solution). The resulting solution (100 mg/mL) was acidic (pH, approx 2.0) and was diluted with sterile water to achieve a pH of 6 as determined by litmus paper, which resulted in a solution with an orbifloxacin concentration of 50 mg/mL.

For each rabbit, a 24-gauge catheter was placed in a marginal ear vein and secured with tape for IV administration of orbifloxacin. The catheter was flushed with approximately 0.5 mL of heparinized saline (0.9% NaCl) solution before and after orbifloxacin administration to ensure delivery of the entire calculated volume. The orbifloxacin was injected slowly over approximately 1 minute, and the catheter was removed after being flushed with heparinized saline solution.

Sample collection
Venous blood samples (1 mL) were collected into microtainer tubes containing lithium heparin by standard venipuncture techniques from the right or left saphenous or jugular vein of each rabbit at 0 (immediately), 0.25, 0.5, 1, 2, 4, 6, 12, and 24 hours after oral and IV administration of orbifloxacin. The blood samples were centrifuged at 2,000 X g for 5 minutes immediately after collection, and the plasma from each sample was decanted and stored at –70°C until analysis.
Determination of plasma orbifloxacin concentration

All assays were developed, performed, and validated in the Pharmacology Core Laboratory at Colorado State University. Orbifloxacin was measured in samples by means of high-performance liquid chromatography–tandem mass spectrometry with difloxacin as an internal standard. The system consisted of a binary liquid chromatography pump coupled to a triple quadrupole mass spectrometer. Chromatographic separation was obtained with a C8 2.5 µm (4.6 × 50 mm) column by use of a liquid chromatography gradient consisting of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). Chromatographic resolution was obtained by holding mobile phase B steady at 12.5% from 0 to 1.75 minutes, increasing linearly to 75% from 1.75 to 2 minutes, holding steady from 2 to 2.5 minutes, decreasing linearly to 12.5% from 2.5 to 3 minutes, and equilibrating at 12.5% from 3 to 3.5 minutes. The liquid chromatography flow rate was 1.3 mL/min, and the sample volume was 10 µL. The analysis run time was 3.5 minutes.

The mass spectrometer settings were optimized for orbifloxacin, and the mass spectrometer was run in multiple-reaction monitoring mode with positive electrospray ionization monitoring ion transition m/z of 396.2 to 352.2 for orbifloxacin and m/z of 400.3 to 356.3 for difloxacin. No interfering peaks were detected at the monitored ion transitions in extracted blank rabbit plasma.

Orbifloxacin concentrations were calculated by use of linear regression analysis of peak area ratios (ie, orbifloxacin area vs difloxacin area) and extrapolation from calibration curves generated in blank rabbit plasma fortified with increasing concentrations of orbifloxacin. A 14-point calibration curve ranging from 1 ng/mL to 8 µg/mL was generated and split into 2 individual curves to accommodate the large range of concentrations in the unknown samples. The lower concentration calibration curve was linear (r = 0.996) between 1 ng/mL and 2.5 µg/mL and contained 4 sets of quality control samples at 2.5 ng/mL, 100 ng/mL,

### Table 1—Pharmacokinetic parameters for orbifloxacin following oral or IV administration of 10 (n = 3) or 20 mg/kg (n=3) once to healthy purpose-bred adult female New Zealand White rabbits (Oryctolagus cuniculus).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oral administration</th>
<th>IV administration</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10 mg/kg</td>
<td>20 mg/kg</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</td>
<td>1.66 ± 0.51</td>
<td>3.00 ± 0.97</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–t&lt;/sub&gt; (µg*h/mL)</td>
<td>9.57 ± 1.5</td>
<td>175 ± 1.2</td>
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<tr>
<td>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (µg*h/mL)</td>
<td>10.5 ± 1.9</td>
<td>19.1 ± 2.1</td>
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<td>AUMC (µg*h&lt;sup&gt;2&lt;/sup&gt;/mL)</td>
<td>53.8 ± 19.9</td>
<td>107.4 ± 29.2</td>
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<tr>
<td>Cl (mL/min*kg)</td>
<td>—</td>
<td>—</td>
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<tr>
<td>λ&lt;sub&gt;12&lt;/sub&gt; (µg/mL)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V&lt;sub&gt;dss&lt;/sub&gt; (L/kg)</td>
<td>6.0 ± 1.3</td>
<td>5.7 ± 0.29</td>
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<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>2.0</td>
<td>3.3 ± 1.1</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>8.1 ± 1.7</td>
<td>8.4 ± 2.4</td>
</tr>
<tr>
<td>MAT (h)</td>
<td>5.6</td>
<td>5.8</td>
</tr>
<tr>
<td>λ&lt;sub&gt;β&lt;/sub&gt; (h&lt;sup&gt;–1&lt;/sup&gt;)</td>
<td>0.081 ± 0.001</td>
<td>0.097 ± 0.015</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2α&lt;/sub&gt; (h)</td>
<td>7.3 ± 1.1</td>
<td>8.6 ± 0.55</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2β&lt;/sub&gt; (h)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>k&lt;sub&gt;12&lt;/sub&gt; (h&lt;sup&gt;–1&lt;/sup&gt;)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>k&lt;sub&gt;21&lt;/sub&gt; (h&lt;sup&gt;–1&lt;/sup&gt;)</td>
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<tr>
<td>k&lt;sub&gt;10&lt;/sub&gt; (h&lt;sup&gt;–1&lt;/sup&gt;)</td>
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<tr>
<td>k&lt;sub&gt;22&lt;/sub&gt; (h&lt;sup&gt;–1&lt;/sup&gt;)</td>
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<tr>
<td>A (µg/mL)</td>
<td>—</td>
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<td>B (µg/mL)</td>
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<td>α (h&lt;sup&gt;–1&lt;/sup&gt;)</td>
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<tr>
<td>β (h&lt;sup&gt;–1&lt;/sup&gt;)</td>
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<td>β</td>
</tr>
<tr>
<td>F (%)</td>
<td>49.5 ± 5.0</td>
<td>44.8 ± 1.4</td>
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</tbody>
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Each rabbit was administered the assigned dose orally and then IV following a 1-week washout period. For all parameters except bioavailability, values represent the mean ± SD.

A, B = Coefficients of the biexponential equation used to describe the drug disposition curve. AUC<sub>0–t</sub> = Area under the plasma concentration-time curve from administration to the last sample collection time (24 hours). AUC<sub>0–∞</sub> = Area under the plasma concentration-time curve from administration to infinity. AUMC = Area under the first moment of the plasma concentration-time curve. Cl = Clearance. F = Systemic bioavailability. k<sub>12</sub> = Elimination rate constant from the central compartment. k<sub>13</sub> = First-order rate constant associated with the transfer of unbound drug between the central and peripheral compartments. k<sub>11</sub> = First-order rate constant associated with the transfer of unbound drug between the peripheral and central compartments. MAT = Mean absorption rate. MRT = Mean residence time. t<sub>1/2α</sub> = Terminal half-life for the first-order rate constant associated with the distribution phase of the drug disposition curve. t<sub>1/2β</sub> = Terminal half-life for the first-order rate constant associated with the elimination phase of the drug disposition curve. V<sub>dss</sub> = Apparent volume of distribution based on the AUC. V<sub>darea</sub> = Apparent volume of distribution at steady state. α, β = Exponents of the biexponential equation used to describe the drug disposition curve. λ<sub>α</sub> = Terminal rate constant. — = Not calculated.
and 2.5 µg/mL. The high concentration curve was linear \((r = 0.995)\) between 25 ng/mL and 8 µg/mL and contained 4 sets of quality control samples at 100 ng/mL, 2.5 µg/mL, and 5 µg/mL. In addition, seven 1:2 dilution standards were made ranging from 1 to 25 µg/mL, with dilution quality control samples at 2.5, 10, and 15 µg/mL. Accuracy and precision of both calibration curves was within 15%, accuracy of all quality control samples was within 10%, and precision was within 5% for all concentrations. The intra-run coefficient of variation was 7.2%.

Plasma samples for unknowns and calibration curves were prepared for analysis via protein precipitation by use of acetonitrile with 0.1% formic acid. Briefly, rabbit plasma (100 µL) was added to 1.5-mL Eppendorf tubes containing 10 µL difloxacin (final concentration, 100 ng/mL) as an internal standard. To each tube, 200 µL of acetonitrile with 0.1% formic acid was added followed by vortex mixing for 10 minutes. Samples were then centrifuged at 17,000 X g for 10 minutes. Following centrifugation, 200 µL of the supernatant was diluted with 200 µL of water and transferred to glass autosampler vials. Early time point–high concentration samples were diluted 1:2 with blank rabbit plasma prior to extraction to bring concentrations into the linear portion of the calibration curves.

**Pharmacokinetic analysis**

Pharmacokinetic parameters were calculated with commercial software. Data obtained following IV administration of orbifloxacin were analyzed by noncompartmental and compartmental analyses. A 2-compartment model with 1/Y weighting provided the best fit for the data. Data obtained following oral administration of orbifloxacin were analyzed by non-compartmental analysis, and only the \(C_{max}\), \(t_{max}\), elimination rate constant, terminal half-life, AUC, area under the first moment curve, mean residence time, and mean absorption time were calculated for that route of administration. Systemic bioavailability was calculated as the \((AUC_{oral} X \text{dose}_{oral})/(AUC_{IV} X \text{dose}_{oral})\).

**Results**

All rabbits received 100% of the assigned oral dose of orbifloxacin, and no adverse effects were observed following orbifloxacin administration regardless of the dose or route of administration. The pharmacokinetic parameters (Table 1) and plasma orbifloxacin concentration over time following oral and IV orbifloxacin administration at both doses (Figure 1) were summarized. Mean plasma orbifloxacin concentration remained above 0.1 µg/mL (the MIC for many pathogens) for 12 and 24 hours after oral administration of the drug at 10 and 20 mg/kg, respectively.

**Discussion**

To our knowledge, the present study was the first to assess the pharmacokinetics and bioavailability of orbifloxacin following oral and IV administration at doses of 10 and 20 mg/kg in rabbits. Results of another study indicate that parenteral administration of orbifloxacin to rabbits at a dose of 5 mg/kg failed to achieve therapeutic concentrations; therefore, we chose to evaluate higher doses (10 and 20 mg/kg) of the drug in the present study. The pharmacokinetics of orbifloxacin has been previously evaluated in dogs, cats, horses, Japanese quail, rats, and rabbits.

The bioavailability of oral orbifloxacin is nearly 100% in dogs and quail because the drug is readily absorbed following oral administration. In the present study, the mean bioavailability of orbifloxacin in rabbits was 100%.
bits following oral administration ranged from 46.5% to 52.5%, which was similar to that (68%) estimated for horses.17 Both rabbits and horses are hindgut fermenters, so it was not surprising the bioavailability of orbifloxacin was similar between those 2 species but differed substantially from that for non–hindgut fermenters such as dogs and quails.

Other antimicrobials such as enrofloxacin3 and florfenicol4 also have lower bioavailability in rabbits than in other species. The natural physiology of rabbits requires them to forage and consume frequent meals, which decreases the gastrointestinal transit time (and thus the time available for gastrointestinal absorption of orally administered drugs), compared with that of other species. For drugs with limited bioavailability following oral administration, increasing the dose may help offset the limited gastrointestinal absorption but should only be done with caution because higher doses of a drug may adversely affect the intestinal microflora. In the present study, the bioavailability of orbifloxacin was determined following administration of the assigned dose only once. Further studies in which rabbits are administered the assigned dose of orbifloxacin multiple times are necessary to better elucidate the drug’s mechanism of absorption and safety in this species.

In the present study, the mean t\textsubscript{max} of orbifloxacin following oral administration was 2 hours for the 10 mg/kg group and 3.3 hours for the 20 mg/kg group, which was similar to the t\textsubscript{max} reported for other fluoroquinolones in various species.6,12 The mean terminal half-life of orbifloxacin for the rabbits of the present study ranged from 7.3 to 8.6 hours, which was substantially longer than that for dogs19 (4.32 hours) and horses20 (5.08 hours). The mean volume of distribution of orbifloxacin at the steady state was 1.4 and 1.3 L/kg following IV administration of 10 and 20 mg/kg, respectively, to the rabbits of the present study, which was consistent with that following parenteral administration of orbifloxacin at a dose of 5 mg/kg (1.71 L/kg)18 or other fluoroquinolones (1 to 2 L/kg)6 to rabbits, and parenteral administration of orbifloxacin at a dose of 2.5 mg/kg (1.61 L/kg) to dogs.19 Moreover, the mean C\textsubscript{max} and AUC for the rabbits in the 20 mg/kg group were nearly twice the mean C\textsubscript{max} and AUC for the rabbits in the 10 mg/kg group, which suggested that orbifloxacin has dose linearity in rabbits.

Fluoroquinolones are currently dosed on the basis of the AUC-to-MIC ratio, in which the AUC during a 24-hour period is compared with the MIC of the target pathogen.21,25–28 The MIC is the lowest concentration of an antimicrobial that will inhibit the growth of a particular bacterium during a 24-hour period. In human medicine, the recommended dose for a fluoroquinolone is that expected to achieve an AUC of 100 to 125 times the MIC for the target pathogen at the site of infection.29–31 However, the threshold for the AUC-to-MIC ratio may vary by pathogen,6 and that recommendation was made on the basis of study results that involved severely ill or immune-compromised individuals that likely required higher drug doses than those required by most rabbits.

It has been suggested that fluoroquinolone doses that achieve an AUC of 30 to 60 times the MIC for the target pathogen might be adequate to treat susceptible infections in immunocompetent patients.6,32 Ultimately, the efficacy of an antimicrobial is dependent on the in vivo interactions among the drug, pathogen, and patient’s immune system.

Unfortunately, we could not account for the protein-binding capacity of orbifloxacin in the present study because, to our knowledge, no studies have been performed to evaluate the protein binding of orbifloxacin in rabbits, and that was beyond the scope of this study. Protein binding varies by drug and species. Results of a recent study33 indicate that the fluoroquinolone thymoquinone has > 99% protein binding in rabbits, whereas other fluoroquinolones such as ofloxacin and levofloxacin have < 10% protein binding in rabbits.34 Because we did not determine the free fraction of orbifloxacin in the plasma of the rabbits of the present study, we cannot recommend a dose for use of the drug in rabbits given that the dose will vary depending on the extent of protein binding by the drug.

The safety of orbifloxacin was not evaluated in the present study and, to our knowledge, has not been evaluated in any other study. One of the authors (MSJ) of this study has successfully treated rabbits with susceptible infections with orbifloxacin at a dosage of 20 mg/kg, PO, once daily for 7 to 21 days on a routine basis without any adverse effects. Although anecdotal evidence suggests that treatment of rabbits with orbifloxacin at 20 mg/kg, PO, once daily on a short-term basis is not associated with toxicosis and only a single dose of the drug was administered to the rabbits of the present study, long-term fluoroquinolone use has been associated with substantial adverse effects such as changes in immature articular cartilage, arthropathy, and neuropathies in rabbits and other species.35–37 Clinical manifestations of arthropathy may resolve after cessation of fluoroquinolone use;6,36 however, results of another study38 indicate that joint lesions may persist for at least 5 months after cessation of fluoroquinolone use in growing dogs.

Results of the present study provided pharmacokinetic data for orbifloxacin in rabbits when administered at 10 and 20 mg/kg by oral and IV routes and suggested that the pharmacokinetic properties of orbifloxacin were similar to those of other fluoroquinolones in rabbits. Further studies are necessary to determine the protein-binding activity of orbifloxacin in rabbits before a dose of the drug can be recommended for that species.

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Footnotes
a. Orbax oral suspension (30 mg/mL), Merck Animal Health, Summit, NJ.
References


